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Histopathology of rainbow trout gills after exposure to copper

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Abstract: Copper is a very common element in water resources. For this reason, concerns about the risks and consequences of this element in water contamination are arising. The purpose of this study was to investigate the effects of water-born CuO (Copper (II) oxide) on the rainbow trout, *Oncorhynchus mykiss* gill tissue to establish a suitable biomarker for copper in water resources. Samples with 18±3g body weight were exposed to CuO for a week with 0.0125, 0.037, 0.075, and 0.15 ppm of copper sulfate and a control group (without CuO). Physicochemical properties of water were 15±2ºC, pH 7-8, $Caco_3$ 270mg/l and oxygen saturation 90.9±0.2%. At the end of 7 days, 9 fish were caught randomly from each treatment and second gill from left side of fish were removed for histological study. Hyperplasia, oedema, epithelial lifting in secondary lamella and lamellar aneurysm were observed in gill tissues, showing that CuO, as a copper ion, has significant adverse effects on gill tissues of rainbow trout and gills can be a suitable biomarker for copper in water resources.

Keywords: Biomarker, Gill, Heavy metals, *Oncorhynchus mykiss*.

Introduction

Copper (Cu) is a very common element with a ubiquitous distribution in the environment and biota (Carbonell & Tarazona 1994). Copper is an essential micronutrient naturally occurring in unpolluted fresh waters, in concentrations ranging from 0.2 to 30mg/l (USEPA 2007). Although copper is essential to animals and higher plants in low amounts (e.g., cytochrome c oxidase and superoxide dismutase) (Zhou & Gitschier 1997), hazard and pathological effects of copper is reasonably well-known in fish in excess amounts (Grosell et al. 2007; Mustafa et al. 2012). Copper causes destructive effect on the ultrastructure of the pavement cells (PVC) and chloride cells (CC) of fish gill epithelia such as lifting, rupture peeling of lamellar epithelium, lamellar fusion, hyperplasia, and cellular hypertrophy (Pelgron et al. 1995; Dang et al. 2000; Mazon et al. 2002). However, the severity of these damages depends on the species' sensitivity, the copper concentration, and the exposure time.

Along with the development of industries, a progressive decline in the water quality occurred resulting in poor ecological condition of the water bodies. Chemical analyses may not be sufficient to properly assess the adverse effects of the complex mixture of water contaminants (He et al. 2011). Thus, to assess the destructive effects of pollutant in water bodies, a biomarker-based bio-monitoring is a promising approach to provide early warning signs of exposure (Viarengo et al. 2000; Au 2004; Zorita et al. 2007; Tlili et al. 2010; Pereira et al. 2013). According to Water Framework Directive (WFD), fish represent one of the key elements to evaluate the rivers

ecological conditions (Scardi et al. 2008; Hermoso et al. 2010). Fish gills are the first target of pollutants because of their large surface area, quick absorption and direct contact with the external environment (Pandey et al. 2008). For this reason, fish gills are suitable organs for toxicology studies.

Analyzing the histopathological changes in gill tissue has been an instrument widely used in aquatic toxicology to bio-monitor acute and chronic situations (Stentiford et al. 2003; Van Dyke & Pieterse 2008; Pereira et al. 2013). Changes in gill epithelium are a consequence of a range of contaminant exposures, with the severity of changes depending on the pollutant concentration and exposure period (Santos et al. 2011). Therefore, the aim of this study was to evaluate gill histopathology of rainbow trout after exposure to copper sulfate. Rainbow trout, *Oncorhynchus mykiss*, is an economically important species commercially farmed in many countries throughout the world. It is exotic to Iranian inland waters which introduced to the Tigris River, Caspian Sea, Lake Urmia, Namak Lake, Kavir, Esfahan and Kor River basins (Esmaeili et al. 2014) and has a rapid growth that can easily adapt to environmental conditions. Finding data on environmental biomarker for this species can be useful in aquatic toxicity management and environmental safety.

Materials and methods

One hundred and fifty-five live specimens of rainbow trout were obtained from local farms in October 2013. Samples weighted 18±3g in average. They were acclimatized in an aquarium (30×20×35cm; 2100 liter) for one week in the Gonbad Kavous University Fisheries Laboratory. Fish were divided into five groups (three replicates) with 10 individuals in each aquarium and were treated with 0.00 (control group), 0.0125, 0.037, 0.075, and 0.15ppm of copper sulfate. These sub-lethal concentrations were determined according to ATSDR (1990). It is noted that ATSDR, determined lethal concentration of copper 1-8ppm for freshwaters fishes. No feed was given during the experiment (one week). There were no significant differences between the aquaria in water quality in the following parameters: pH: 7±0.004; temperature: 15±2˚C; hardness: 270±0.05ppm and oxygen saturation: 90.9±0.2%. The photoperiod was 12h light and 12h dark.

After one week, nine fish were caught randomly from each aquarium and the second gill arch from left side of each fish was separated for histopathological studies and fixed in 10% formalin solution and dehydrated in 96% ethanol, cleared with xylene, impregnated with paraffin, embedded, sectioned, mounted, and stained with Haematoxylin and Eosin (H&E) (Bucke 1972). Histopathological changes induced by treatments in the tissues were photographed using Nikon photomicroscope.

Results

Gill morphology of rainbow trout was normal in all the unexposed control samples. Exposure to copper sulphate caused gill injuries such as Hyperplasia (shortening, rounding and fusion of the secondary lamellae), epitthelial hypertrophy (lifting cell layer by oedematous), lamellar fusion (coupling between two adjacent secondary lamellar epithelium) and lamellar aneurism (shutting capillary path within the secondary lamellae) (Fig. 1). Hyperplasia and swollen mucocytes was the most observed injuries. Table 1 shows type of injuries with damage degree. The results showed that hyperplasia increased until 0.037ppm copper concentration and decreased in

Table 1. Rainbow trout gill damage during the lethal concentrations of copper sulfate.

Types of injuries	Sub-lethal concentrations (ppm)				
	Control	0.0125	0.037	0.075	0.15
Hyperplasia	-	+	+++	+	-
Lamellar fusion	-	-	-	+++	+
Lamellar aneurism	-	-	-	+	++
Leukocyte infiltration	-	-	+	-	+++
Epithelial hypertrophy	-	+++	++	+	+

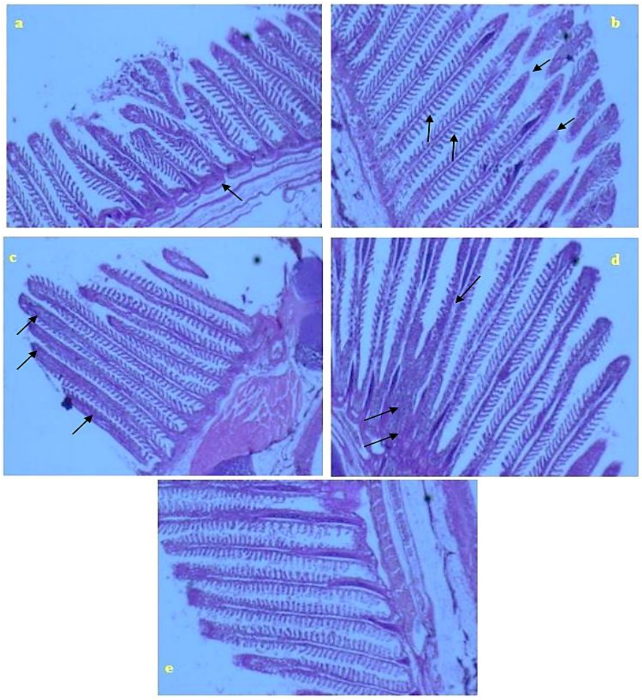

Fig.1. Microphotographs of gill histopathological changes by copper sulfate in Rainbow trout (a-d) and control group (e). Epithelial hypertrophy (a), Hyperplasia (b), lamellar fusion and swollen mucocytes (c) and lamellar aneurism and oedema (d).

0.075ppm. Lamellar aneurism and leukocyte infiltration increased in 0.15ppm copper concentration while hyperplasia, lamellar fusion and epithelial hypertrophy decreased (Fig. 1).

Discussion

Copper is a well-known respiratory and ionoregulatory toxicant in fish (Handy 2003; Grosell et al. 2007; Al-Bairuty et al. 2013). Mucus secretion is often the first line of defense to metal exposure in the gills. This phenomenon can temporarily protect gill tissue from injuries (Handy & Maunder 2009). Mucus secretion and swollen mucocytes were observed in this study, but it seems to be not sufficient to protect the gills from the injury causing by copper sulfate. Although hyperplasia and lamellar fusion known to be induced by many gill tissue irritants, however, focal points of cellular hypertrophy and necrosis followed by epithelial rupture, reflect the direct deleterious effects of heavy metals in fish gills (Mazon et al. 2002). In addition, similar gill injuries by Titanium (TiO_2) in *Cyprinus carpio* (Hao et al. 2009) and Silver in *Danio rerio* (Griffitt et al. 2009) were reported. Further, the types of gill injuries by copper evaluated in this study also have been reported by other researchers (Monteiro et al. 2008; Al-Bairuty et al. 2013; Pereira et al. 2013).

Rainbow trout gill had various reactions in different copper doses. In 0.0125ppm, slight hyperplasia and sever epithelial hypertrophy was observed. This reaction shows that the fish immune system is ready to respond to contaminant. Epithelial thickening was attributed to the appearance of macrophages and other leucocytes integrated in a compensatory response of tissue repair (Teh et al. 1997). Monteiro et al. (2008) reported that hyperplasia and epithelial hypertrophy were clearly related to low copper doses and lifting was not significantly correlated with gill copper deposition at high copper doses. They also declared that lamellar fusion occurs in chronic copper exposure which coincided with our findings.

Monteiro et al. (2008) stated that oedema and aneurisms were clearly related to short-term copper exposure, lamellar fusion with chronic copper exposure, lifting with increasing time at the lower metal concentrations and changes in filament epithelium thickness with the higher metal concentrations. According to Mallatt (1985), gill lesions can be divided into two groups; one that reflects the direct effect of toxicants and another one that corresponding to defense responses of fishes. Pereira et al. (2013) stated that filament epithelium proliferation and necrosis were the biological responses that contribute mostly to discriminate the ecological status classification. Several studies have been demonstrated, on laboratory experiments, to determine the toxicity of heavy metals, organochlorine pesticides and petroleum hydrocarbon products to fish gill (Al-Attar, 2007; Garcia-Santos et al. 2007; Patnaik et al. 2011; Santos et al. 2011; Hesni et al. 2011; Moitra et al. 2012), and many researchers used fish gill as a tool to assess the presence of contaminants, in natural aquatic systems

(Stentiford et al. 2003; Fernandes et al. 2007). Fernandes et al. (2007) previously stated that lesions severity and extension might be an accurate indicator of the toxicant levels, but the extent and severity of each particular lesion is variable among different species.

This study showed that gill biological responses could be suggested as a useful biomarker to identify ecosystems perturbation. However, reaction to water contaminations is varying for each species; but the main injuries in each group of pollutants (such as metals, pesticides, herbicides etc.) are constant. In general, each biological response reflects the degree of water contamination.

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